CAGER R PACKAGE

Exploring all its (current) functions

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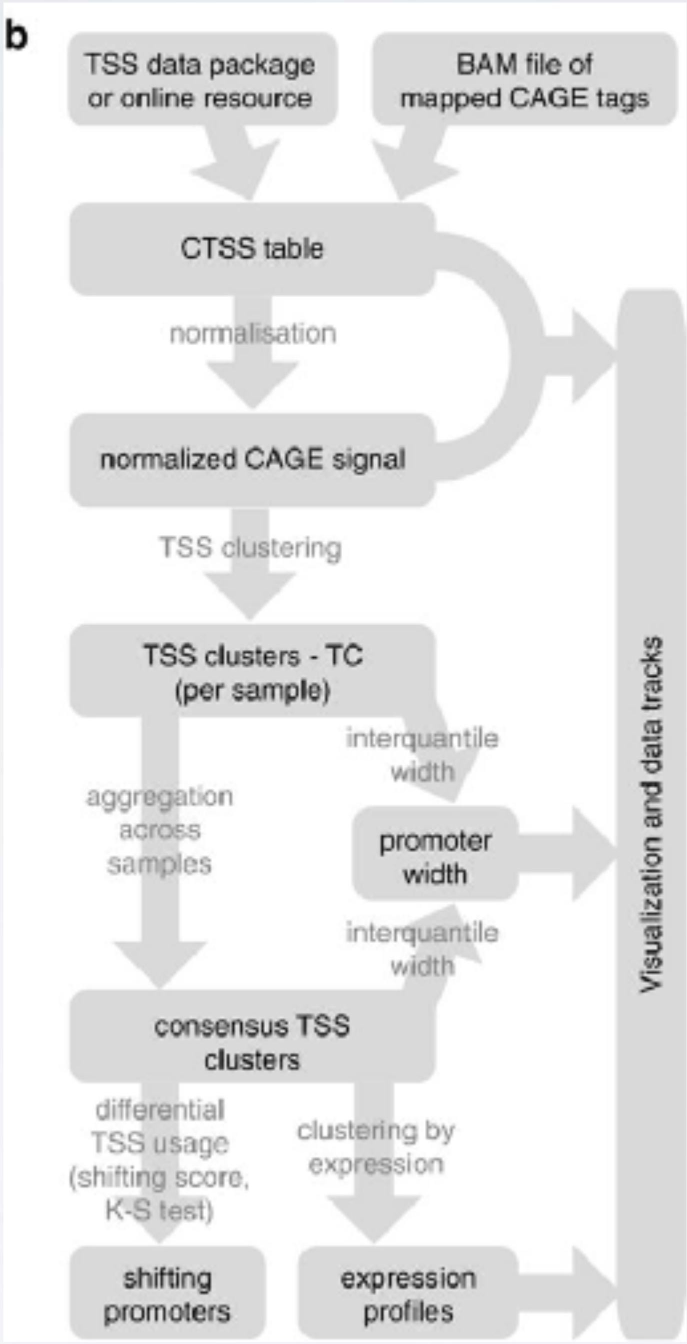
OVERVIEW

the next ~30 minutes

CAGEr Analysis

- Import F6 style format and creating the CAGEr object
- Normalisation & QC
- Tag clusters & consensus clusters
- Global expression patterns
- Promoter shifting
- Create tracks
- Create dinucleotide heatmaps

CAGER WORKFLOW



Haberle V, _et al_. CAGEr: precise TSS data retrieval and high-resolution promoterome mining for integrative analyses. Nucl Acids Res 2015;43(8):e51.

STARTING POINT

I prepared some code

this is found at: https://github.com/leonieroos/CAGEr-F6-workshop

Download the whole dir and save

- open the directory and go to tutorial and open the .rmd file into R studio

DATA FORMATS

CAGEr accepts multiple formats

I CAGE tags mapped to genome

- BAM & BED files

II CTSS files

- Tab separated files with genomic coordinates and number of tags for each CTSS

III CAGE datasets from R packages

- FANTOM5/4/3

From all these, we can create a **CAGEset object**

This is the basis from which the CAGEr functions all work

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CREATING A CAGESET

Let's start!

Fantom6 Data is in BED Format.. but not *one row per tag*

Easily solved by selecting the columns of:

chromosome, end, strand, and column number 5 (amount of tags per position)

CTSS file

chr1	101	+	5
chr1	104	+	2

line 53

CREATING A CAGESET

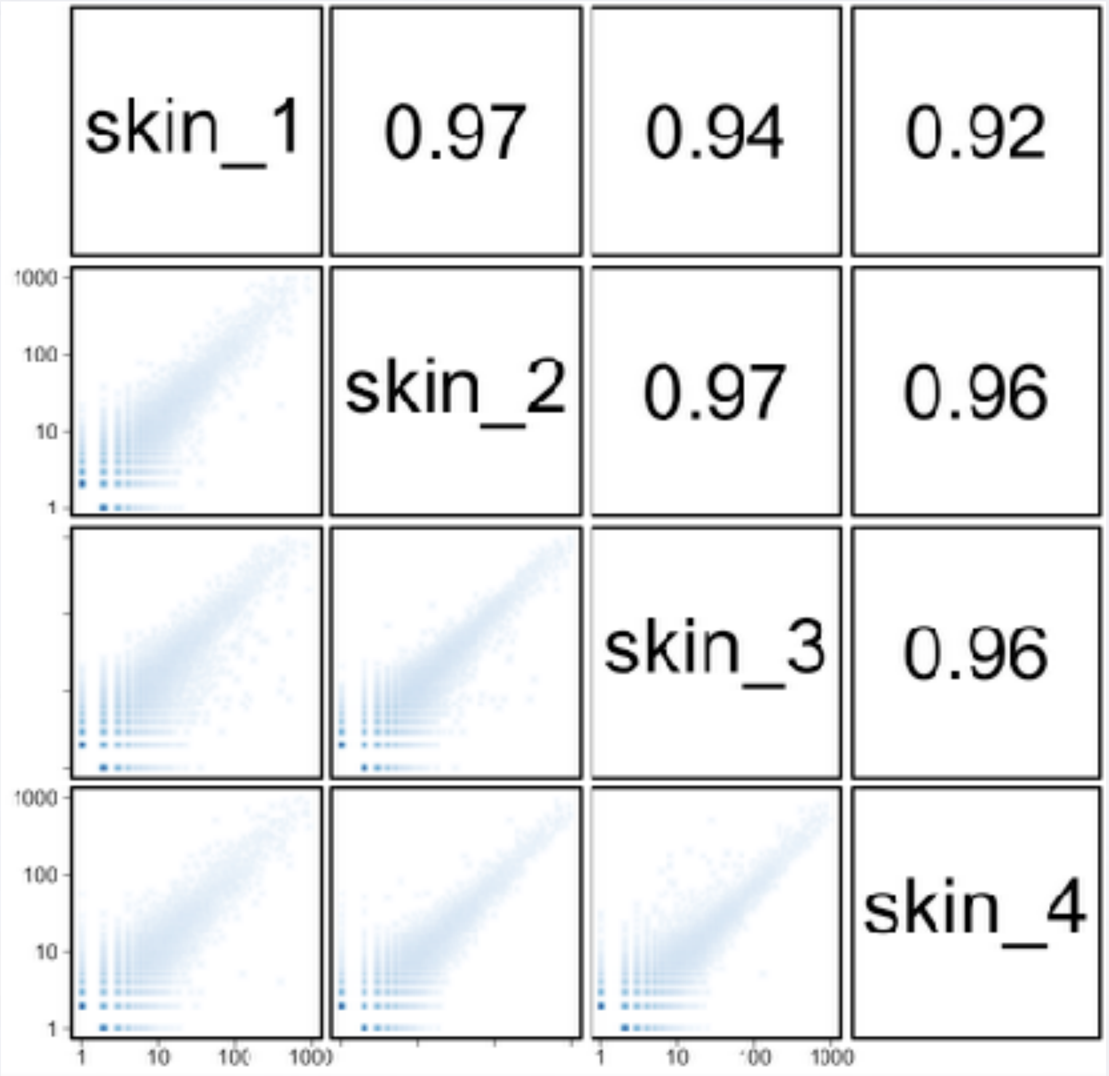
Let's start!

```
### load the CAGEr package
library(CAGEr)
### BSgenome with the right version
library(BSgenome.Hsapiens.UCSC.hg38)

### define where the ctss.bed files provided are located for CAGEr
# where the files can be found
pathsToInputFiles <- list.files("../data/ctss_tables", full.names = TRUE)

### creating a CAGEset object
myCAGEset <- new("CAGEset", genomeName = "BSgenome.Hsapiens.UCSC.hg19", inputFiles = path
sToInputFiles, inputFilesType = "ctss", sampleLabels = paste("skin_",1:4, sep = ""))

# you can check the object:
myCAGEset
```

```
### reading in the data
getCTSS(myCAGEset)
# get a dataframe of ctss counts:
ctss <- CTSStagCount(myCAGEset)
head(ctss)
```

CORRELATION BETWEEN SAMPLES

```
### creating a correlation plot and table of the samples
corr.m <- plotCorrelation(myCAGEset, samples = "all", method = "pearson")
```



NORMALISATION

Library size differ between samples

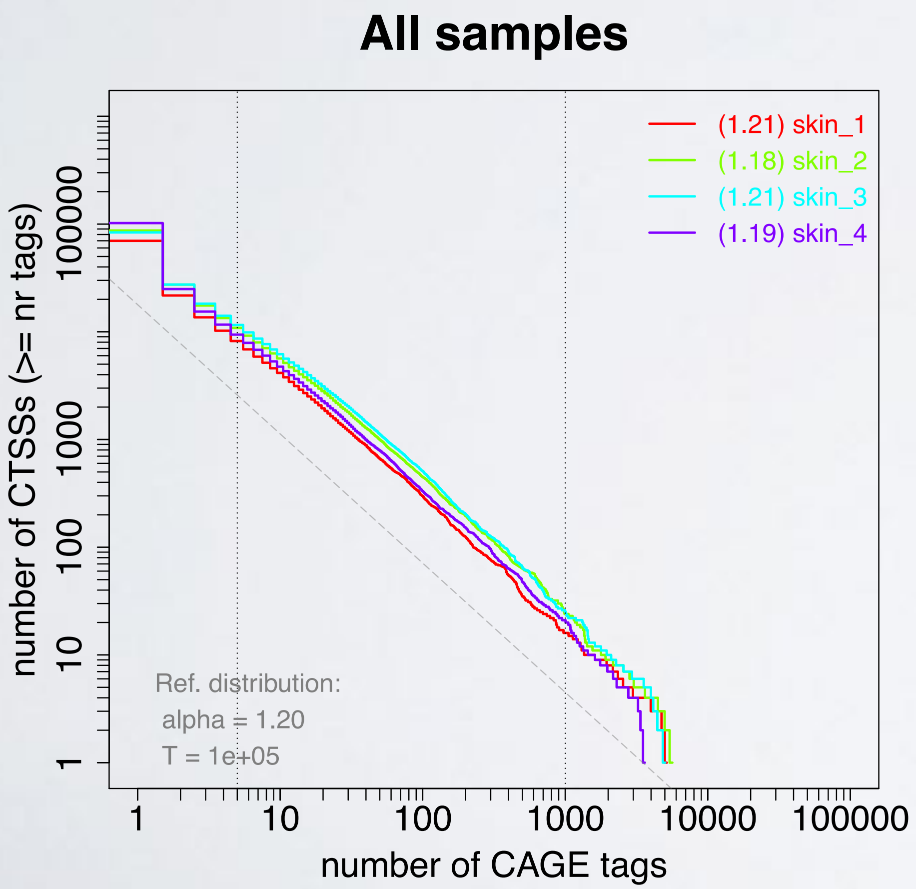
To make samples comparable, we will need to normalise our data.

- Tags per million normalization
- power-law based normalization

Many CAGE-seq data follow a power-law distribution.

NORMALISATION

On a log-log scale this reverse cumulative distribution has a monotonically decreasing linear function



y = -1 * alpha * x + beta

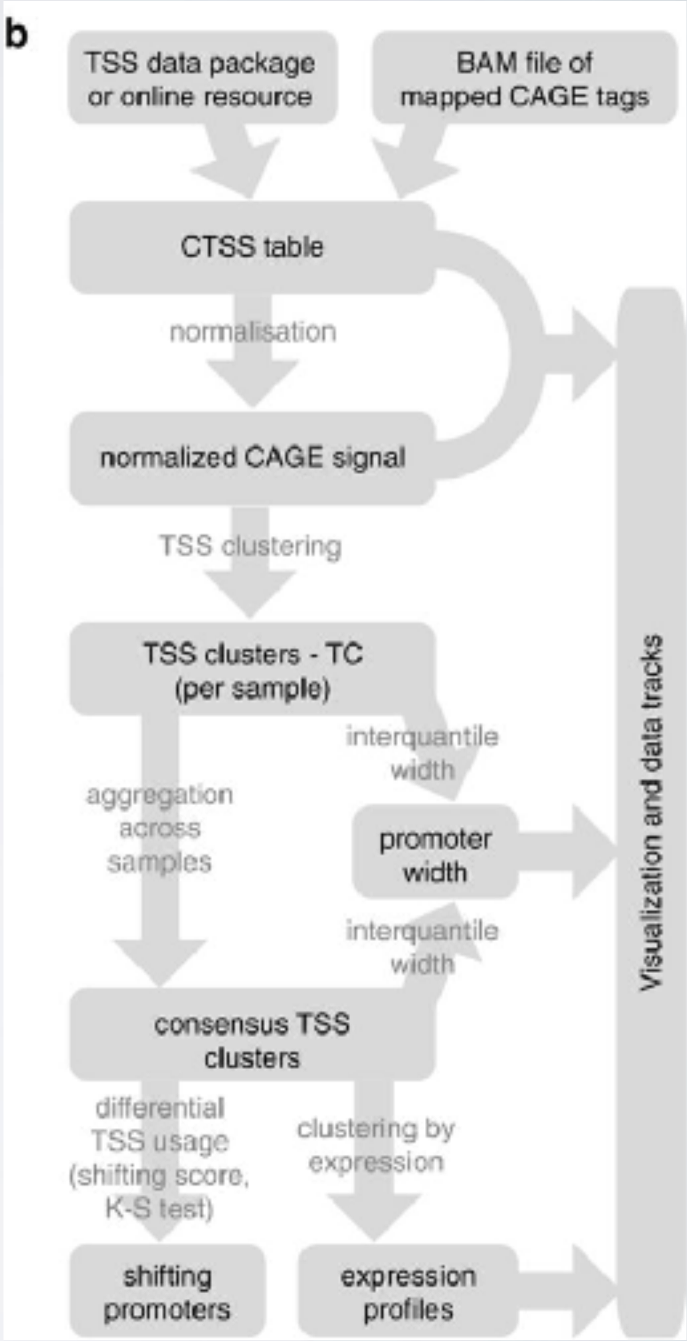
what we need:

- the slope
- the number of tags

Balwierz PJ, Carninci P, Daub CO, et al. Methods for analyzing deep sequencing expression data: constructing the human and mouse promoterome with deepCAGE data. Genome Biology. 2009;10(7):R79.

UP TO NOW

- Imported F6 data into a CAGEset object
- Correlation of CTSS per samples
- Normalised data



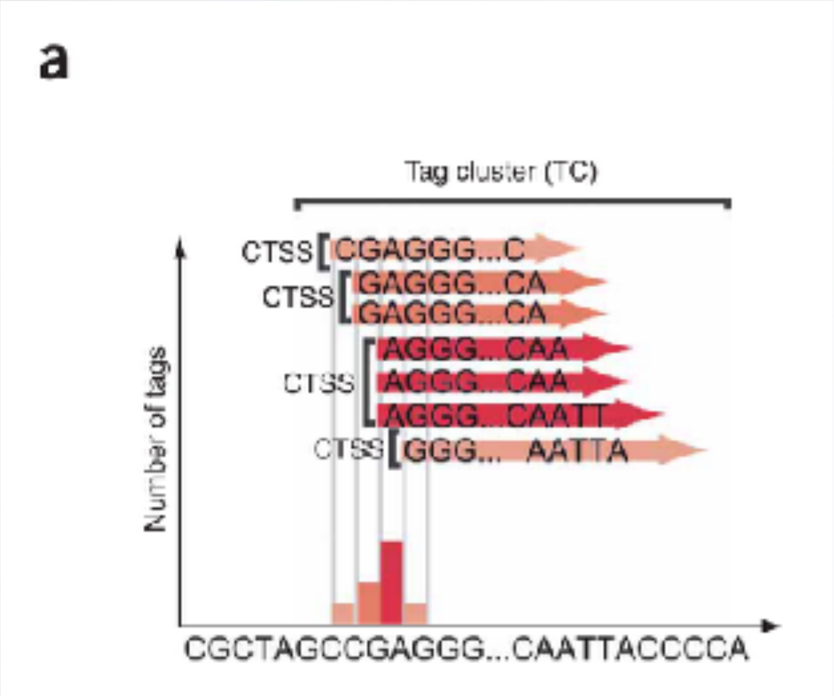
TAG CLUSTERS

CAGE TSS (CTSS)

- CAGE tags with an identical CAGE-tag starting site.

Tag cluster (TC)

- CTSSs in close proximity (same strand)



line 126

Carninci P, _et al_. Genome-wide analysis of mammalian promoter architecture and evolution. Nat Genet 2006;38, 626-635.

TAG CLUSTERS

Tag clusters (TC)

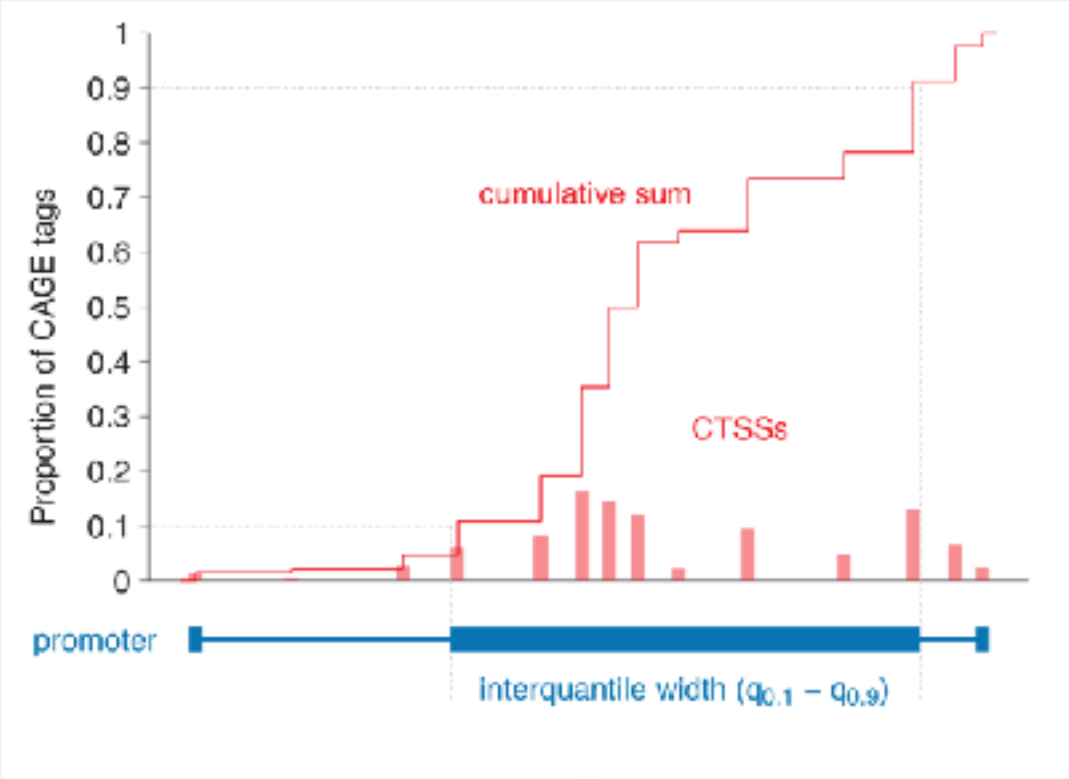
- Max distance between CTSSs is 20 bp
- Single CTSS are allowed if > 5 normalised signal

```
clusterCTSS(object = myCAGEset,
            threshold = 1,
            thresholdIsTpm = TRUE,
            nrPassThreshold = 1,
            method = "distclu",
            maxDist = 20,
            removeSingletons = TRUE,
            keepSingletonsAbove = 5)
```

TAG CLUSTER-WIDTH

Tag clusters (TC)

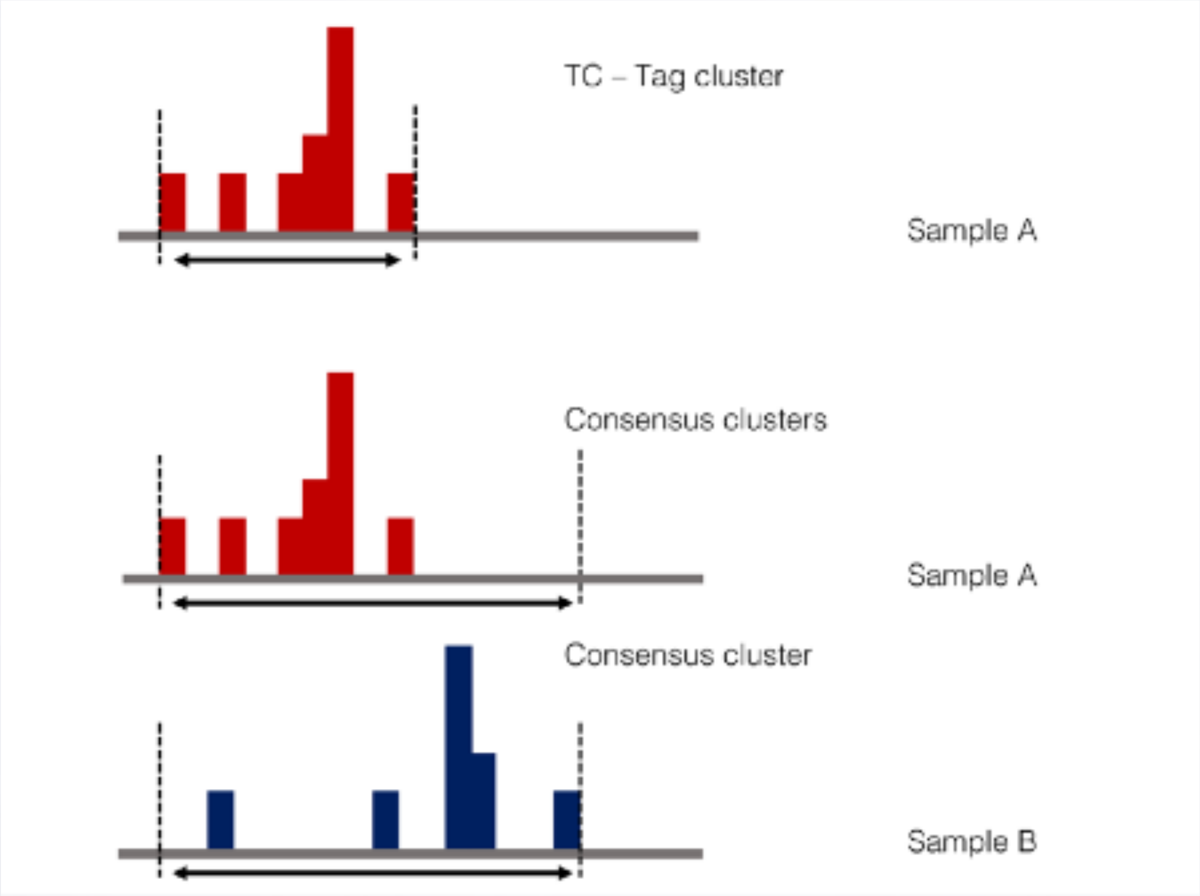
- Width can also be determined by the cumulative distribution between quantiles



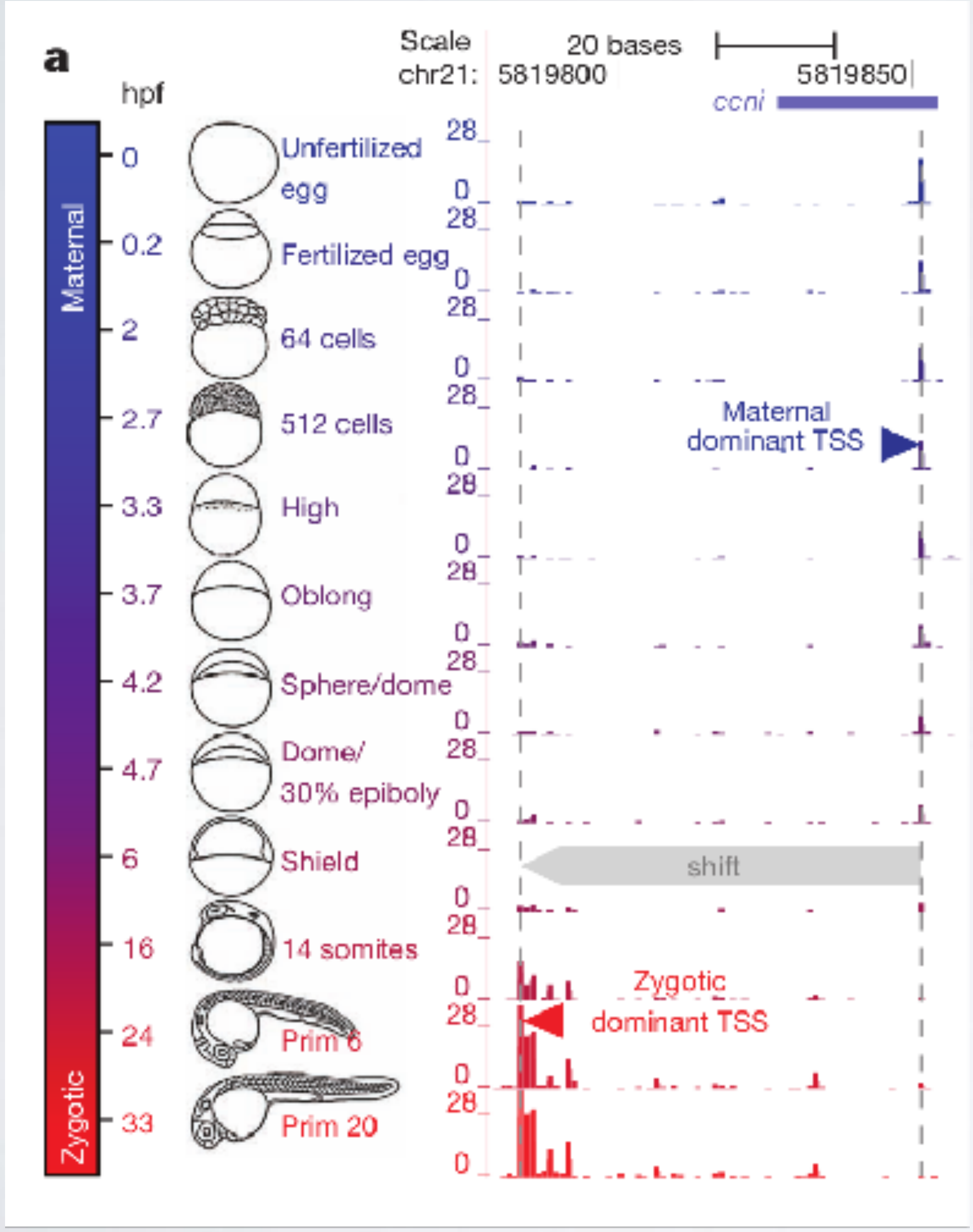
CONSENSUS CLUSTERS

- In many cases TCs do not coincide perfectly
- Maybe two TCs in one sample and one large in other

what if you want to compare TC clusters across samples?



PROMOTER SHIFTING

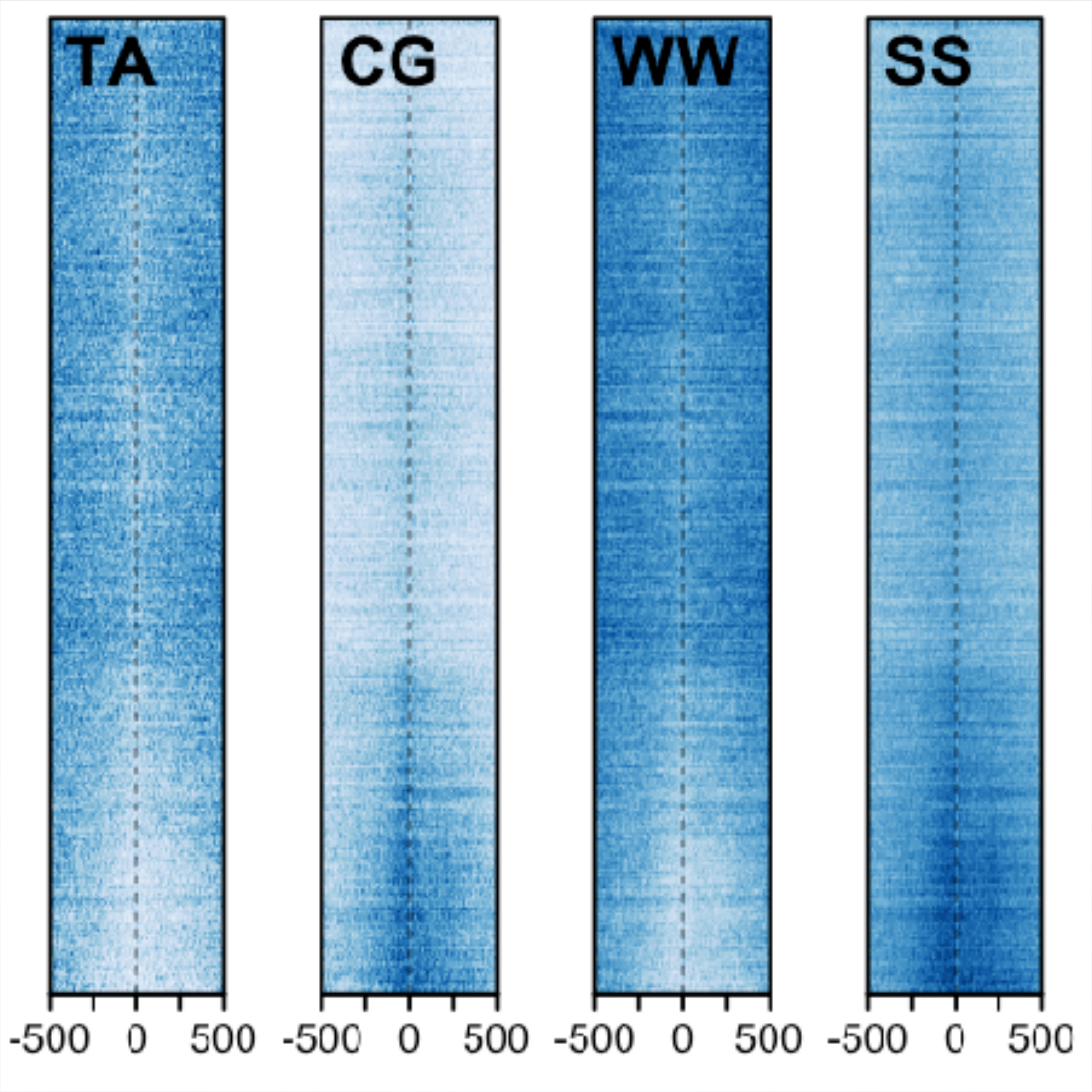


This method has also been implemented in CAGEr

Haberle V, _et al_. Two independent transcription initiation codes overlap on vertebrate core promoters. Nat 2014;507(7492):381-385.

DOMINANT TSS

cluster	chr	start	end	strand	nr_ctss	dominant_ctss	tpm	tpm.dominant_ctss	q_0.1	q_0.9	interquantile_width
1	chr3	3126907	3127018	+	46	3126949	233.2163609	63.32701553	3126937	3126963	27



PROMOTER SHIFTING

The method from haberle *et al* (2014) has also been implemented in CAGEr

- Shifting is detected using cumulative distribution per sample CAGE signal
- A shifting score is determined by the difference in cumulative distributions
- A Kolmogorov-Smirnov is performed to give a general assessment of differential TSS usage